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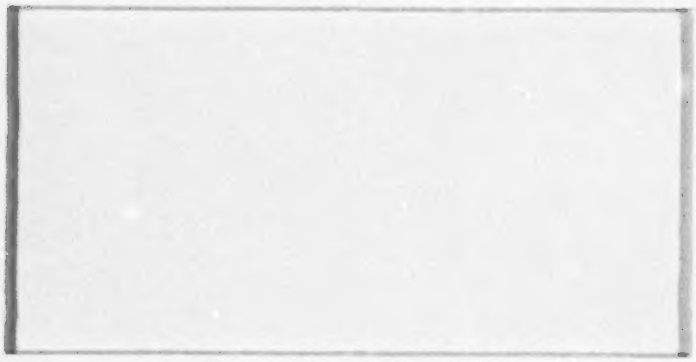

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UPTAKE AND TRANSLOCATION OF RADIOACTIVELY LABELLED HERBICIDES IN A 1:1 MIXTURE OF 2,4-D AND 2,4-DP APPLIED TO THE FOLIAGE OF WHITE ASH AND CAROLINA POPLAR

Report No 83-537-K

M. Kirby
Biologist
Biological Research Section
Chemical Research Department

ABSTRACT

The uptake and translocation of radioactively labelled herbicides applied in a 1:1 mixture of 2,4-D and 2,4-DP to the foliage of white ash, a resistant species, and Carolina poplar, a sensitive species, were investigated. The distribution of the radioactivity in the treated trees suggested that the resistance of ash was attributable to inadequate translocation of herbicide to the roots and that the sensitivity of poplar was attributable to translocation of only one component of the mixture, 2,4-D, to the roots.

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740634-649-031	834.1	January 13, 1984	83-537-K

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EXECUTIVE SUMMARY

UPTAKE AND TRANSLOCATION OF RADIOACTIVELY LABELLED HERBICIDES IN A 1:1 MIXTURE OF 2,4-D and 2,4-DP APPLIED TO THE FOLIAGE OF WHITE ASH AND CAROLINA POPLAR

M.K. Kirby
Biological Research Section
Chemical Research Department

Management of the vegetation on rights-of-way is required to prevent trees from growing into the power lines and causing outages. At present, herbicides are the most reliable means of vegetation control, and although they are economical relative to other methods, high costs are involved due to the necessity of retreating rights-of-way with herbicide every four to seven years. Retreatment is necessary to control trees which have re-established from seed and to control the growth of suckers and sprouts which developed from trees which were previously treated but not killed by herbicides. The suckers and sprouts grow much faster than trees establishing from seed because their root system is already well developed. It is inescapable that retreatment will be required under management schemes relying on herbicides alone due to seed invasion and establishment. However, the length of time between treatments could be increased substantially and the costs reduced, if better control of existing trees on rights-of-way were achieved.

At present a 1:1 mixture of the phenoxy herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4-DP (2,4-dichlorophenoxypropionic acid) is used throughout most of southern Ontario for the control of broad-leafed deciduous species. The mixture is formulated at 700 g active ingredient per litre and is usually applied as a spray to the foliage of trees after dilution with water to form a 1% solution. White ash is resistant to this mixture, and frequently suckers prolifically following treatment. Carolina poplar can similarly sprout prolifically, but is generally more sensitive to 2,4-D and 2,4-DP than ash. Reasons which could account for the difference in the sensitivity of these species to 2,4-D and 2,4-DP include differences in

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herbicide uptake, translocation and metabolism. In this study herbicide uptake and translocation were investigated in ash and poplar using mixtures in which one or other of 2,4-D and 2,4-DP were radioactively labelled. Metabolic studies were not undertaken and the conclusions reached were based on the distribution patterns of the radioactivity in the treated trees.

The distribution of radioactivity in the treated trees indicated that although 2,4-D and 2,4-DP were applied in a 1:1 mixture, they were not taken up nor translocated in equal amounts. The main direction of translocation of both herbicides in poplar was away from the roots (ie upwards from the area of herbicide application), while in ash there was no preferential direction of movement. The resistance of ash is likely due to a lack of translocation of phytotoxic quantities of herbicide to the roots, while the sensistivity of poplar is likely due to translocation of phytotoxic quantities of only one component of the mixture (2,4-D) to the roots. These conclusions should be substantiated by indentifying the radioactive compounds. The role of 2,4-DP with respect to its influence on the uptake and translocation of 2,4-D should be assessed, since 2,4-DP did not appear to be responsible for the toxicity of the mixture of 2,4-D and 2,4-DP to poplar.

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To Mr. F.J. Kee
Director of Research

UPTAKE AND TRANSLOCATION OF RADIOACTIVELY LABELLED HERBICIDES IN A 1:1 MIXTURE OF 2,4-D and 2,4-DP APPLIED TO THE FOLIAGE OF WHITE ASH AND CAROLINA POPLAR

1.0 INTRODUCTION

Management of vegetation on rights-of-way is necessary to prevent trees from growing into power lines and causing outages. The currently available management techniques fall into three broad categories, namely: mechanical methods such as cutting; chemical methods such as herbicide application; and biological methods such as planting species on rights-of-way which are thought to inhibit tree growth. At present, herbicide application is the most widely used means of vegetation control on rights-of-way, and although the method is relatively economical, high costs are involved due to the necessity of regularly retreating rights-of-way with herbicide every four to seven years. Part of the need for retreatment arises from the re-establishment of trees from seed, and part from growth of suckers and sprouts from trees that were previously treated but not killed by herbicides. The suckers and sprouts often number as many as eight or nine per treated tree and characteristically grow faster than trees establishing from seed because they have a well developed root system. The need for retreatment cannot be completely eliminated by herbicide use since tree establishment from seed would continue to pose problems, but retreatment would be required less frequently if herbicides were completely effective in killing treated trees.

Since 1979, Ontario Hydro has used a 1:1 mixture of the phenoxy herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4-DP (2,4-dichlorophenoxypropionic acid) to control broad-leafed deciduous species on rights-of-way in southern Ontario. The mixture is generally formulated at 700 g active ingredient per litre (Ontario Hydro General Specification for Control of Vegetation, Insects and Fungi) and is usually applied as a spray to the foliage of actively growing trees after dilution with water to form a 1% solution. White ash (Fraxinus americana L.) is a species which is resistant to this herbicide combination and frequently suckers prolifically following treatment. Carolina poplar (Populus x canadensis Moench) can similarly sucker prolifically, but is more sensitive to the mixture of 2,4-D and 2,4-DP than ash.

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In a previous experiment using greenhouse-grown trees, the response of ash and poplar to foliar applications of a 1:1 mixture of 2,4-D and 2,4-DP was compared at final application concentrations ranging from 0.25 to 3 times the concentration normally used by Ontario Hydro (Ontario Hydro Research Division Report No 83-448-K). Volatilization of herbicide in that experiment altered the level of exposure of the treated trees somewhat, but it was still apparent that ash is tolerant to a wide range of concentrations of 2,4-D and 2,4-DP. Only the two highest concentrations of applied herbicide resulted in the death of the foliage of ash while none of the concentrations produced death of the roots. In contrast, all concentrations were lethal to the foliage of poplar and resulted in the death of the roots of some of the treated trees. However, since root death did not occur in all poplars treated with any concentration, 2,4-D and 2,4-DP cannot be considered entirely satisfactory as a toxicant for this species.

Explanations which could account for the relative sensitivity of poplar as compared to ash and the variability in the sensitivity of individual poplar trees include differential herbicide uptake, differential herbicide translocation and differential herbicide metabolism. These factors have been shown to account for differences in the herbicide sensitivity of several herbaceous and woody species (see, for example, Mayeux and Scifres 1980, Bovey and Mayeux 1980, Wyrill and Burnside 1976), but have not been investigated with respect to the response of ash and poplar to 2,4-D and 2,4-DP. This study was undertaken to determine the uptake and translocation of 2,4-D and 2,4-DP after application to the foliage of ash and poplar. The herbicides were applied in a mixture in which one of the two was radioactively labelled. The patterns of uptake and translocation were determined by assaying various plant tissues for radioactivity.

2.0 METHODS AND MATERIALS

2.1 Plant Propagation

Two year old white ash and Carolina poplar were provided by the Ontario Ministry of Natural Resources and were planted in plastic pots (25 cm in diameter) in April. Both species broke dormancy almost immediately. The soil consisted of a mixture of two parts loam to one part peat moss. A nutrient analysis performed by the Department of Land Resources at the University of Guelph revealed that the soil was deficient in phosphorus. Superphosphate at a rate of 500 g/m^3 was added to the soil mixture to bring the phosphorus to recommended levels. The trees were randomly arranged in the greenhouse and were maintained by

regular watering and application of the fertilizer 20-20-20 (NPK) (obtained from Plant Products, Bramalea, Ontario). The soil at the surface of the pots was hand-raked twice a month to prevent caking and provide aeration for the roots.

2.2 Herbicide Application

The trees were treated with either a combination of an unlabelled mixture of 2,4-D and 2,4-DP and labelled 2,4-D, or the unlabelled mixture and labelled 2,4-DP. The procedures adopted for herbicide application were designed to simulate field methods, and to minimize the possibility of environmental contamination by the radioactive material. The herbicide was applied in a two-step procedure in which an unlabelled 1:1 mixture of 2,4-D and 2,4-DP was sprayed to drip point on all poplar leaves and ash leaflets,[1] except those which were to be treated with the radioactively labelled herbicides. The leaves treated with the unlabelled mixture were allowed to dry before the radioactive material was applied to the remaining leaves and leaflets.

The mixture of the unlabelled butoxyethanol esters of 2,4-D and 2,4-DP was obtained from Union Carbide, Chatham, Ontario (Desermone 700, batch No S77123). It contained 350 g/L ai (active ingredient) of 2,4-D and 370 g/L ai of 2,4-DP (Ontario Hydro Research Division Report No CB81-10-H). These values are within the range of concentrations of herbicide batches accepted for field use (see, for example, Ontario Hydro Research Division Reports No CB82-1-H and CS80-1477-K). The herbicide was diluted with water to yield a 1% solution before application to the experimental trees. Spraying took place in August using a small hand sprayer equipped with a Tee Jet nozzle 8004 and set at pressure of 30 psi. The herbicide was applied to a total of 40 trees of each species removed from the greenhouse. The soil in the pots was covered with plastic sheeting during spraying to prevent herbicide contact with the soil. The leaves or leaflets which were to be treated with the radioactively labelled herbicides were enclosed in whirlpak bags during the spraying.

Uniformly ^{14}C ring labelled butoxyethanol esters of 2,4-D and 2,4-DP were prepared by Dr. B.E. McCarry of McMaster University, Hamilton, Ontario. The specific activity of the compounds and the total activities supplied are listed in Table 1.

---

[1] Ash has compound leaves and poplar has simple leaves. Compound leaves consist of a blade which is divided into separate portions called leaflets. There are no divisions of the blade of a simple leaf. Ash leaves may have from 5 to 11 leaflets, but commonly have 7.

TABLE 1

ACTIVITIES OF THE RADIOACTIVELY LABELLED HERBICIDES

Labelled Compound	Specific Activity	Total Activity
2,4-D	21 mCi/mmol	128 µCi
2,4-DP	20 mCi/mmol	39 µCi

Each of the radioactive compounds was diluted with the unlabelled mixture of 2,4-D and 2,4-DP and then applied as drops to the upper surface of four leaves of each poplar and four terminal leaflets of each ash. The high specific activity of the labelled 2,4-D and 2,4-DP allowed the compounds to be added to the mixture of 2,4-D and 2,4-DP without significantly altering the 1:1 ratio in the final solution. The leaves and leaflets which were treated with the mixtures containing the radioactive compounds were located approximately halfway along the main stem allowing a distinction to be made between upward (acropetal) and downward (basipetal) movement of herbicide. Each leaf or leaflet received one 10 µL drop dispensed from an Ependorff pipette. The drops were spread across the surface of the leaf or leaflet. Each tree received a total of 1 µCi of labelled 2,4-DP or 2 µCi of labelled 2,4-D. Fifteen trees of ash and poplar were treated with the herbicide mixture containing the labelled 2,4-DP and 25 of each species were treated with herbicide mixture containing the labelled 2,4-D. Five trees of each species were used as controls to determine background levels of radioactivity. These were maintained in a separate compartment in the greenhouse to avoid the possibility of damage due to volatilization (Ontario Hydro Research Division Report No 83-448-K).

2.3 Sampling of Plant Tissues and Recovery of the Radioactivity

Sampling was conducted on the first, third, seventh, fifteenth and fortieth days after treatment. Three labelled 2,4-DP treated trees of each species and five labelled 2,4-D treated trees of each species were sampled on each occasion. The control trees were sampled only on the last day of the experiment. In the discussions which follow, the terms 'treated leaves' and 'treated leaflets' will refer to those leaves and leaflets which received the application of radioactively labelled herbicides. The poplar were divided into the following sections:

- leaves above the treated leaves
- treated leaves

- leaves below the treated leaves
- secondary roots

Ash were harvested in a similar manner, except that the treated leaves were sorted into those leaflets which had received the radioactively labelled herbicide and those which did not.

All leaves or leaflets treated with the radioactively labelled herbicides, except those sampled on the fortieth day after treatment, were washed in 50 mL of 95% methanol for 2 min prior to freezing to remove the unabsorbed herbicide. The treated leaves and leaflets were too brittle by the last day to be washed. All samples were placed in whirlpak bags and frozen until the time of analysis.

The efficiency of the leaf washing technique was tested by applying the mixtures containing the radioactively labelled herbicides to leaves or leaflets of extra trees and by allowing them to dry for approximately 1 h and then washing them twice in methanol. If the leaf wash technique were effective, all the applied radioactivity should be recovered in methanol washes of these samples.

The radioactivity in the samples was measured by combustion followed by liquid scintillation counting. The leaf samples were homogenized in distilled water using a Virtis Homogenizer and then freeze-dried. A subsample weighing from 0.25 to 1 g was pressed into a pellet and combusted in a Packard Tri Carb 306C oxidizer programmed for a 2 to 3 min burn time. The $^{14}CO_2$ was absorbed in an organic base, Carbo-Sorb, to which an equal amount of scintillation cocktail, Permafluor V, was added. Both Carbo-Sorb and Permafluor are products of the Packard Instrument Company, Downers Grove, Illinois. The final volume of the sample was dependent on the weight of material combusted and followed Packard Instrument Company recommendations. The samples were assayed for radioactivity in a Packard Tri Carb 300 Scintillation Counter. The counts per minute were converted to pCi or μCi after correction for background (1 pCi = 1 x 10^{-6} μCi).

The root samples were processed by freeze-drying a subsample, making it into a pellet, and combusting the pellet in the same manner described for leaf tissue. The leaf washes were processed by combusting and assaying a 100 μL subsample for radioactivity.

3.0 RESULTS AND DISCUSSION

3.1 Radioactivity Recovered in the Leaf Washes and From the Treated Leaves and Leaflets

The methanol washes were not effective in removing all the applied radioactivity from the leaf surfaces at the end of the first hour after herbicide application (Table 2). The average recovery in all treatments was less than 52% of the applied dose in the first wash and less than 7% in the second. The low additional amount recovered in the second wash indicates that most of the radioactivity that could be recovered by this technique had been recovered in the first wash. A higher overall recovery had been anticipated in view of other studies in which alcohol washes were successfully used to remove unabsorbed phenoxy herbicides from leaf surfaces (Pallas 1963, Wyrill and Burnside 1976, Sharma and Vanden Born 1970, Soteres et al 1983). No explanation can be offered for the ineffectiveness of the technique in the present study.

Since the unabsorbed herbicide could not be accurately measured, the quantities of radioactivity found in the leaf washes were added to those recovered from the treated leaves and leaflets to give a total for the treated areas. Data for the first and fortieth days after treatment is shown in Table 3. The recovery of radioactivity from the trees treated with labelled 2,4-D was high in all cases, accounting for a minimum average of 60.9% of the applied dose. The mean radioactivity recovered from the poplar leaves treated with labelled 2,4-D was less than from the ash leaflets on both days, while for both species the mean recovered on the fortieth day was less than on the first. The temporal decrease in the amount of radioactivity recovered reflects losses of herbicide due to volatilization and export from the treated leaves.

TABLE 2

RADIOACTIVITY RECOVERED FROM METHANOL WASHES OF POPLAR LEAVES AND ASH LEAFLETS ONE HOUR AFTER HERBICIDE APPLICATION

HERBICIDE APPLIED	SPECIES	REPLICATE	WASH	RADIOACTIVITY RECOVERED	
				µCi	(% of applied)
2µCi labelled 2,4-D	Ash	1	1	1.291	(64.6)
			2	0.164	(8.2)
			Total	1.455	(72.8)
2µCi labelled 2,4-D	Ash	2	1	0.747	(37.4)
			2	0.094	(4.7)
			Total	0.841	(42.1)
1µCi labelled 2,4-DP	Ash	1	1	0.269	(26.9)
			2	0.029	(2.9)
			Total	0.298	(29.8)
1µCi labelled 2,4-DP	Ash	2	1	0.225	(22.5)
			2	0.026	(2.6)
			Total	0.251	(25.1)
1µCi labelled 2,4-DP	Poplar	1	1	0.326	(32.6)
			2	0.020	(2.0)
			Total	0.346	34.6
1µCi labelled 2,4-DP	Poplar	2	1	0.384	(38.4)
			2	0.034	(3.4)
			Total	0.418	(41.8)

Note: The leaf washes from poplar treated with labelled 2,4-D were lost before processing.

TABLE 3

THE TOTAL RADIOACTIVITY RECOVERED ONE AND FORTY DAYS AFTER TREATMENT FROM POPLAR LEAVES AND ASH LEAFLETS WHICH RECEIVED APPLICATIONS OF LABELLED 2,4-D OR LABELLED 2,4-DP

HERBICIDE APPLIED	SPECIES	RECOVERY ON DAY 1		RECOVERY ON DAY 40	
		µCi ($\bar{x} \mp$ SD)	(% of applied)	µCi ($\bar{x} \mp$ SD)	(% of applied)
Labelled 2,4-D	Ash	1.544 ∓0.124	(77.2)	1.441 ∓0.066	(72.0)
Labelled 2,4-D	Poplar	1.445 ∓0.193	(72.3)	1.219 ∓0.227	(60.9)
Labelled 2,4-DP	Ash	0.332 ∓0.108	(33.2)	0.283 ∓0.029	(28.3)
Labelled 2,4-DP	Poplar	0.395 ∓0.041	(39.5)	0.332 ∓0.054	(33.2)

The radioactivity recovered from the poplar leaves and ash leaflets treated with labelled 2,4-DP was low accounting for a maximum average of 39.5% of the applied dose. The mean radioactivity recovered from the labelled 2,4-DP treated poplar leaves was greater on both sampling days than from the ash leaflets. This may indicate that the uptake of 2,4-DP is greater in poplar than in ash.

The percentage of the applied radioactivity recovered from the leaves and leaflets treated with labelled 2,4-DP was about half of that recovered from those treated with labelled 2,4-D. This suggests that the uptake and translocation of 2,4-DP by ash and poplar is greater than the uptake and translocation of 2,4-D. A study by Davis et al (1968) similarly suggests that herbicides applied in mixtures are not necessarily taken up in the same ratio in which they are applied. Equimolar combinations of

picloram (4-amino-3,5,6-trichloropicolinic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) were applied to the leaves of honey mesquite (Prosopis juliflora (Swartz) DC var glandulosa (Torr.) Cockerell). Greater quantities of 2,4,5-T than picloram were absorbed and translocated so that the ratio of the two herbicides in stem and leaf tissue was not 1:1. Both picloram and 2,4,5-T have a similar mechanism of action to the phenoxy herbicides used in this experiment.

The radioactivity recovered from treated leaves and leaflets was also expressed as a concentration (μCi/g dry weight of leaf tissue). The data are not presented, since the trends were generally similar to those described above. Where discrepancies arose, it was apparent that they were attributable in large part to the fact that the treated leaves and leaflets within a treatment differed in weight, but received the same quantity of radioactive herbicide.

3.2 Radioactivity Recovered from the Remaining Leaflets of the Labelled 2,4-D and Labelled 2,4-DP Treated Leaves of Ash

The remaining leaflets on the treated leaves of ash have no structural counterpart on poplar to allow direct comparisons, but analysis of these leaflets provides information on herbicide distribution and on the rate of herbicide translocation. It is through the area of the remaining leaflets that herbicide must travel before it reaches the stem. The concentrations of radioactivity (pCi/g dry weight of leaf tissue) in the leaflets from the labelled 2,4-DP treated leaves of ash were characterized by the absence of a distinct interpretable temporal trend, largely as a result of the high variability between the replicates of each sampling day (Table 4). However, the data indicate that translocation is rapid, since the mean levels in the leaflets on the first day after sampling do not differ greatly from those found on subsequent days.

Similarly, the concentrations in the leaflets from the leaves treated with labelled 2,4-D exhibit a large variability. However, the mean shows a steady increase from the first to fifteenth day after treatment and a small decrease between the fifteenth and fortieth days. This suggests that translocation of 2,4-D may be more gradual than that of 2,4-DP.

The total quantities of radioactivity recovered from the leaflets on the treated leaves of ash which did not receive an application of labelled herbicide account for up to 4.03% of the applied dose of labelled 2,4-DP, but are generally less than 1%. The total quantities recovered from the labelled 2,4-D treated trees ranged up to 7.3% of the applied dose and averaged 3.8% on the fortieth day after treatment.

TABLE 4

MEAN RADIOACTIVITY (∓SD) RECOVERED FROM THE LEAFLETS OF TREATED LEAVES OF ASH WHICH DID NOT RECEIVE AN APPLICATION OF LABELLED HERBICIDE

(pCi/g dry weight of leaf tissue)

Labelled 2,4-DP

Day 1	Day 3	Day 7	Day 15	Day 40
3 203	2 800	557	2 923	6 143
∓4 646	∓1 115	∓408	∓2 761	∓9 970

Labelled 2,4-D

Day 1	Day 3	Day 7	Day 15	Day 40
8 277	15 857	24 195	34 829	30 463
∓8 080	∓ 7 368	∓16 946	∓40 535	∓17 040

3.3 Concentrations of Radioactivity in the Foliage Above and Below the Treated Leaves

The mean concentrations of radioactivity (pCi/g dry weight of leaf tissue) in the foliage above and below the treated leaves of ash and poplar are shown in Figure 1. There is little difference in the mean concentration of radioactivity in the foliage above and below the treated leaves in both the labelled 2,4-D and the labelled 2,4-DP treated ash on each sampling day, indicating that in ash these compounds move equally well acropetally and basipetally. Temporally, the mean concentration of radioactivity in the upper and lower foliage of the labelled 2,4-DP treated ash remained essentially constant from the first to the fortieth day after treatment. In contrast, the mean concentration in the upper and lower foliage of the labelled 2,4-D treated ash increased five-fold between the first and third day after treatment, but showed little subsequent change. On the first day after treatment the mean concentrations of radioactivity in the upper and lower foliage of the labelled 2,4-D treated ash were approximately equal to those in the labelled 2,4-DP treated ash, but from the seventh to the fortieth day after treatment were a minimum of six times greater. This is

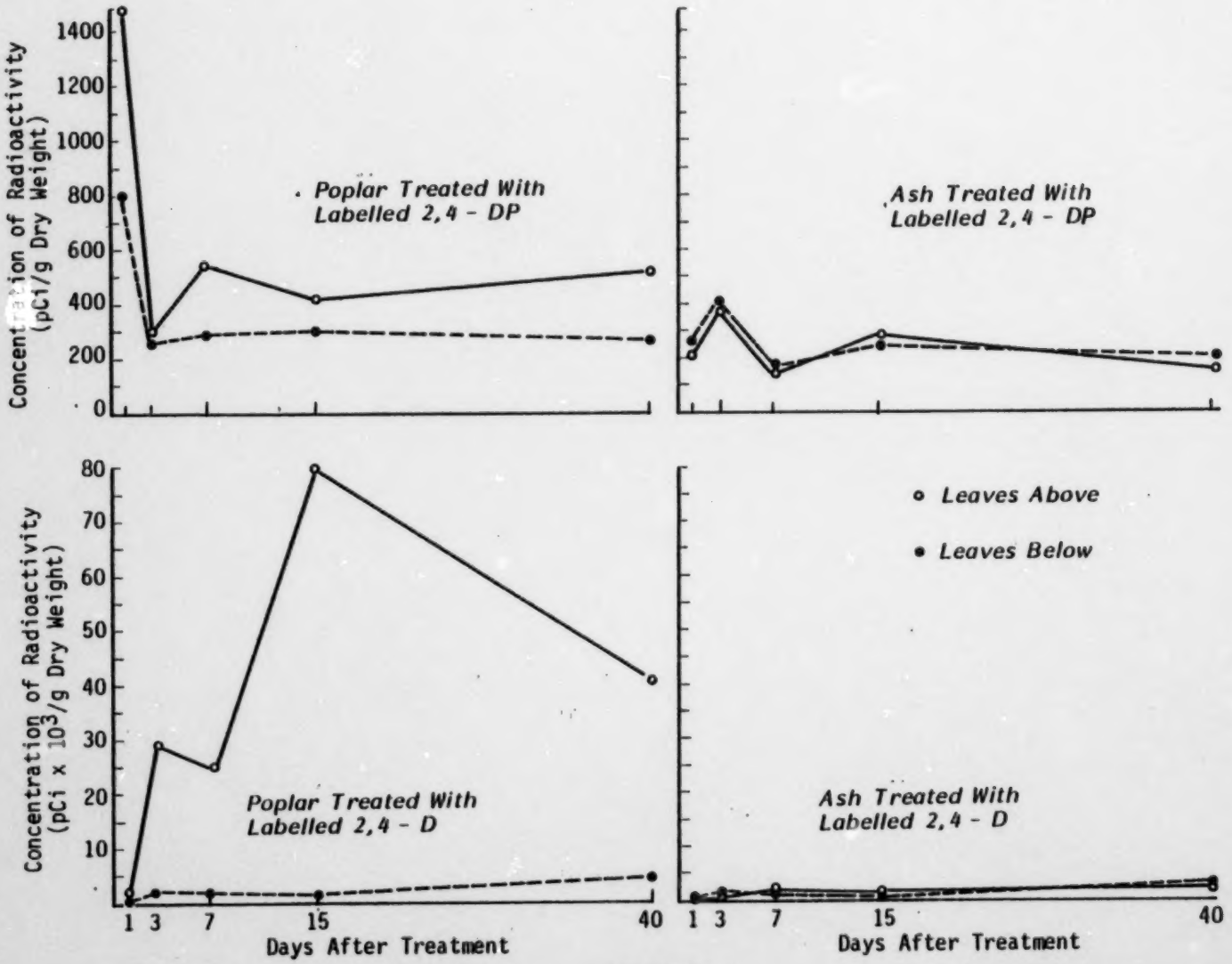

FIGURE 1

MEAN CONCENTRATION OF RADIOACTIVITY IN THE FOLIAGE ABOVE AND BELOW THE TREATED LEAVES

consistent with the earlier suggestion that 2,4-D and 2,4-DP are not taken up and translocated in a 1:1 ratio. If uptake and translocation had occurred in a 1:1 ratio, the concentration of radioactivity in the upper and lower foliage of the labelled 2,4-D treated ash would be expected to be twice that of the labelled 2,4-DP treated ash due to the difference in the amounts of radioactivity initially applied.

The mean concentrations of radioactivity in the lower foliage of both the labelled 2,4-DP and the labelled 2,4-D treated poplar were of the same order of magnitude as in the comparably treated ash. However, the mean concentrations in the upper foliage of the labelled 2,4-D treated poplar were substantially greater than in the ash and than in the lower foliage of the poplar. A similar difference is apparent between the upper and lower foliage of the labelled 2,4-DP treated poplar, but is less pronounced than in the labelled 2,4-D treated poplar. These trends were apparent not only in the mean concentrations but also in the individual values of each replicate. The concentration in the upper foliage of all the labelled 2,4-D treated poplar exceeded that in the lower foliage. The same was true for 75% of the labelled 2,4-DP treated poplar. This would indicate that the predominant direction of movement of 2,4-D and 2,4-DP in poplar was acropetal, rather than basipetal. Eliasson (Eliasson 1965, Eliasson and Hallmen 1973) similarly suggested that 2,4-D moved primarily upwards after foliar application to Populus tremula, a European species of poplar. Preferential upward movement has also been documented for picloram applied with 2,4-DB (4-(2,4 dichlorophenoxy)-butyric acid) to the foliage of bean plants (Phaseolus vulgaris L.)(Hamill et al 1972) and for 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) applied to the stem or bark of red maple (Acer rubrum L.), white ash (Fraxinus americana L.)(Leonard et al 1966) and marabu (Dichrostachys nutans)(Hay 1956, cited in Leonard et al 1966).

3.4 Radioactivity Recovered in the Roots

The mean concentration of the radioactivity in the roots of the labelled 2,4-D treated trees is shown in Figure 2 and for the labelled 2,4-DP treated trees in Table 5. The mean concentrations of radioactivity in the roots of both the labelled 2,4-DP treated ash and poplar were low. There was no discernible temporal trend in the mean concentration of radioactivity in the roots of the labelled 2,4-DP treated ash, but there was a slight increase in the mean concentration in the roots of the labelled 2,4-DP treated poplar between the first and seventh day after treatment. Since radioactivity was detected in the roots on the first day after treatment, translocation of small quantities of herbicide or metabolite occurred very rapidly.

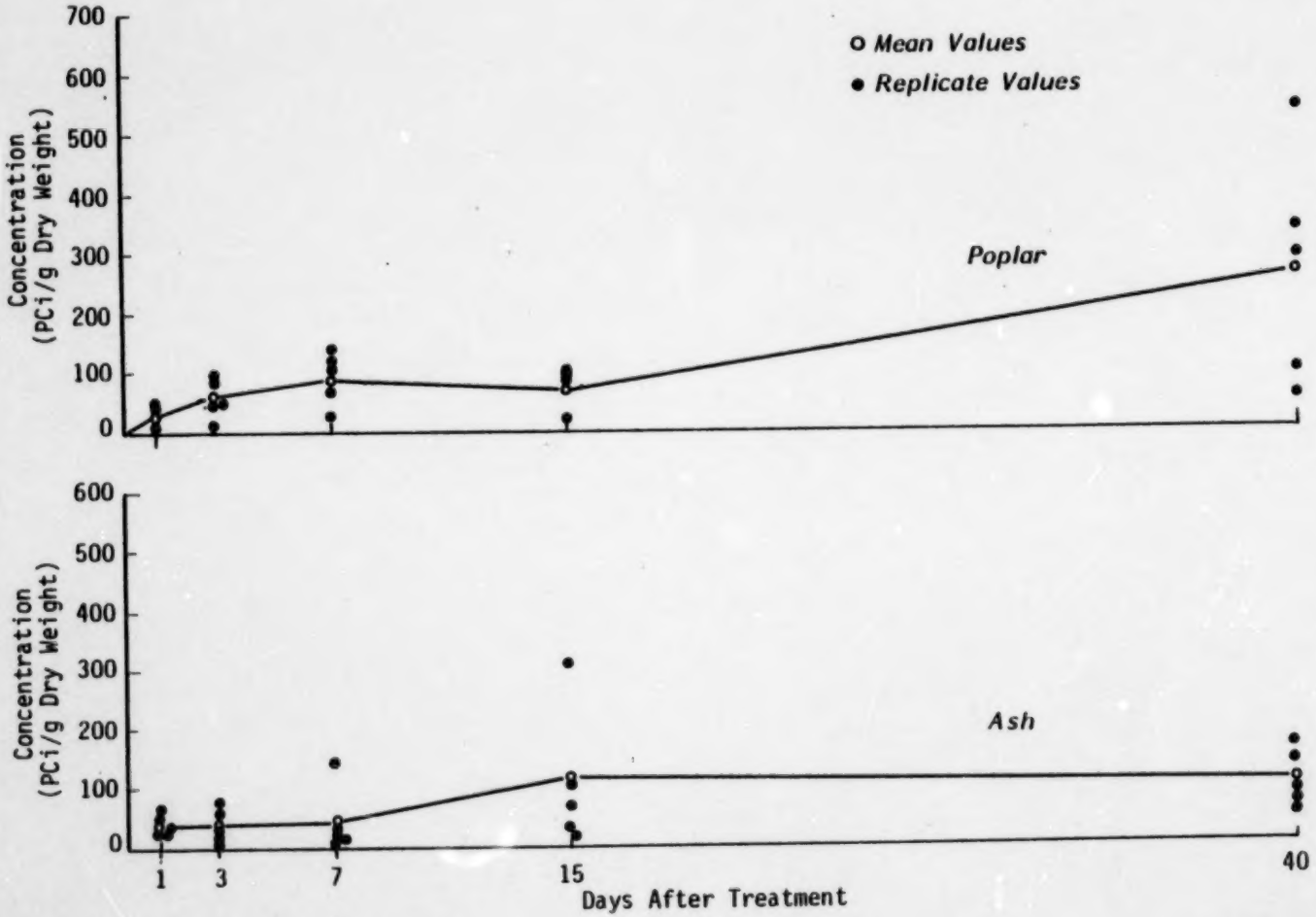

FIGURE 2

CONCENTRATION OF THE RADIOACTIVITY IN THE ROOTS OF THE LABELLED 2,4-D TREATED ASH AND POPLAR

TABLE 5

MEAN CONCENTRATION (∓SD) OF RADIOACTIVITY IN THE ROOTS OF THE LABELLED 2,4-DP TREATED ASH AND POPLAR
(pCi/g dry weight)

Species	Days after Treatment				
	1	3	7	15	40
Poplar	15.05	14.25	34.14	46.37	30.46
	∓ 8.67	∓11.20	∓ 3.02	∓14.40	∓ 5.40
Ash	14.43	13.07	14.33	32.01	14.96
	∓ 7.66	∓ 4.98	∓ 4.99	∓ 9.10	∓ 2.04

The mean concentration of radioactivity in the roots of the labelled 2,4-D treated ash did not differ greatly from those in poplar from the first to the fifteenth day after treatment, but on the fortieth day after treatment the mean concentration of radioactivity in the roots of the poplar was three times higher than that in the ash (Figure 2) and more than eight times higher than that in the labelled 2,4-DP treated poplar. The latter observation is consistent with the earlier suggestion that 2,4-D and 2,4-DP were not taken up nor translocated in the 1:1 ratio in which they were applied. If uptake and translocation had occurred in a 1:1 ratio, the concentration of radioactivity in the roots of the labelled 2,4-D treated trees would be expected to be twice that of the labelled 2,4-DP treated trees due to the difference in the amounts of radioactivity initially applied.

The results of another study of uptake and translocation of 2,4-D and 2,4-DP in poplar and ash are consistent with the relatively high proportion of radioactivity in the roots of the labelled 2,4-D treated poplar (Ontario Hydro Research Division Report No 83-178-K). In that study, measurable quantities of 2,4-D were found in the roots of poplar but not in ash. No 2,4-DP was detected in the roots of either species. The lack of detection of 2,4-DP in the roots of ash and poplar and the lack of detection of 2,4-D in the roots of ash in that study may indicate that the radioactivity is associated with a herbicide metabolite, but is more likely a consequence of the fact that the analytical procedures used were less sensitive than those of the present study.

3.5 Overall Distribution

The results indicate that the pattern of distribution of radioactivity in the treated trees is dependent on both the species and the herbicide under consideration. The differences between the species were more pronounced in the labelled 2,4-D treated trees than in the labelled 2,4-DP treated trees. Similarly, the temporal trends were better defined in the labelled 2,4-D treated trees than in the labelled 2,4-DP treated trees.

As an indication of the fate of the applied herbicide, the distribution of the radioactivity in the foliage of the labelled 2,4-D treated trees on the fortieth day after treatment is shown in Table 6. Most of the radioactivity in both species was recovered from the treated leaves and leaflets. The major sinks for radioactivity in the untreated parts of the foliage were the leaves above the treated leaves in poplar and the untreated leaflets of the treated leaves of ash. The total radioactivity recovered from the roots was not calculated, since only a subsample of the material was dried before combustion. However, the mean concentration of radioactivity in the poplar roots was three times higher than that in the ash on the final day of sampling.

Based on the patterns apparent in the concentration of radioactivity data, the foliar sinks for radioactivity in the trees treated with labelled 2,4-DP are similar to those of trees treated with labelled 2,4-D. However, little radioactivity was present in the roots of either species on the fortieth day after treatment with labelled 2,4-DP, in contrast to the results of the trees treated with labelled 2,4-D. Another difference between the trees treated with labelled 2,4-DP and those treated with labelled 2,4-D lies in the total percentage of the applied radioactivity that was recovered in the foliage on the first day after treatment. Since the time interval since treatment was relatively short it had been expected that most of the applied radioactive dose would have been recovered from the treated leaves and leaflets. However, the amounts recovered from the leaflets and leaves treated with labelled 2,4-DP averaged 33.2% for ash and 39.5% for poplar. The percentages recovered from the untreated parts of the foliage were 0.46% for ash and 0.86% for poplar. The unassayed parts of the trees were the stem tissue and main root. In view of the large percentage of unrecovered radioactivity in the labelled 2,4-DP treated trees, it is possible that one of these parts is a major sink for labelled 2,4-DP.

TABLE 6

DISTRIBUTION OF RADIOACTIVITY IN THE FOLIAGE OF THE LABELLED 2,4-D TREATED TREES ON THE FORTIETH DAY AFTER TREATMENT

Species		Leaves Above Treated Leaves	Leaflets Remaining on Treated Leaves	Treated Leaves and Leaflets	Leaves Below Treated Leaves
Ash	Mean Total Radioactivity Recovered (pCi)	8 106.7	75 922.8	1 440 635.3	3 588.8
	Mean Total as a Percentage of the Applied Dose	0.4%	3.8%	72.0%	0.2%
Poplar	Mean Total Radioactivity Recovered (pCi)	112 242.9		1 219 127.5	29583.0
	Mean Total as a Percentage of the Applied Dose	5.6%		61.0%	1.5%

Using the distribution pattern of radioactivity in the trees treated with labelled 2,4-D as a guide, it is suggested that lack of susceptibility of ash to 2,4-D and 2,4-DP is due to lack of translocation of adequate quantities of herbicide to the roots. Similarly, the resistance of some individual poplar trees to 2,4-D and 2,4-DP may also be due to the same reason, since the concentration in the roots of two of the poplar replicates treated with labelled 2,4-D on the fortieth day after treatment fell within the range of similarly treated ash (Figure 2). Even though relatively large quantities of radioactivity reached the roots of some of the poplar treated with labelled 2,4-D, translocation in this species is apparently mainly upwards and opposite to the direction that would be expected to produce root death, suggesting that 2,4-D and 2,4-DP is an inefficient herbicide for the purpose intended.

These conclusions are based on two assumptions. The first is that the radioactivity is associated with compounds that are phytotoxic. It is expected that at least some of the ^{14}C would be associated with the ester and/or acid forms of 2,4-D and 2,4-DP, based on another study of uptake and translocation of these herbicides in ash and poplar (Ontario Hydro Research Division Report No 83-178-K).[2] It is not known, however, what proportion of the applied herbicide remains as the ester and what proportion is converted to the acid or other metabolites. An interesting result of that experiment was that substantial quantities of the ester form of 2,4-DP were found within the leaves of Carolina poplar and white ash. This is unexpected in view of other reports which indicate that hydrolysis of esters of the phenoxy herbicides to acid forms is rapid (Crafts 1960, Morton et al 1968, Leonard et al 1966). Since there is evidence which indicates that conversion of esters to free acid is necessary for expression of herbicidal activity (Bovey and Young 1980), the lack of appropriate hydrolytic enzymes could represent a mechanism of tolerance. Other reported mechanisms of tolerance to the phenoxy herbicides include detoxification by conjugation of herbicide with plant constituents, hydroxylation of the aromatic rings and alteration of the acetic or propionic side chains (Naylor 1976).

The second assumption on which the conclusions concerning the cause of resistance of ash and some individuals of poplar were based is that poplar roots are of equal or greater sensitivity than ash roots to the quantities of herbicide that the radioactivity detected is presumed to reflect. If ash roots were more sensitive, the levels of herbicide which would be required to kill the roots would be lower than in poplar and would invalidate the rationale behind the conclusion. The concentrations of herbicide that are toxic to roots of any species have never been established and this is a serious drawback of all toxicity studies. The assumption of equal or greater sensitivity of poplar roots to 2,4-D and 2,4-DP than ash roots is not unreasonable in view of the greater sensitivity of the foliage of poplar to lower concentrations of applied herbicide than the foliage of ash (Ontario Hydro Research Division No 83-448-K).

Based on the outcome of this experiment, the following studies would provide further insight into the mechanism of herbicide tolerance and resistance.

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[2] The products were identified only as herbicide in this report, but it was subsequently found that the quantities of 2,4-D detected referred to the total of the acid and ester forms, while the values for 2,4-DP referred only to the ester (G.P. Arron, personal communication).

1. The concentrations of 2,4-D and 2,4-DP that are toxic to roots should be established and a means of enchancing translocation so that toxic concentrations are reached should be sought.

2. The compounds associated with the ^{14}C in this experiment, particularly in the roots of ash and poplar, should be identified.

3. The sinks of 2,4-DP within the ash and poplar should be identified and the role of 2,4-DP with respect to its influence on the uptake and translocation of 2,4-D should be assessed. The quantities of radioactivity found in the roots of poplar and ash after the foliar application of 2,4-^{14}C-DP were low, suggesting that it is unlikely to be responsible for the toxicity of the mixture of 2,4-D and 2,4-DP to poplar. The costs of producing 2,4-DP are higher than for 2,4-D. Equally effective control, at a lower cost, might result if 2,4-D were used alone.

4.0 CONCLUSIONS

1. Although 2,4-D and 2,4-DP were applied in a 1:1 mixture to the foliage of ash and poplar, they were not taken up nor translocated in a 1:1 ratio.

2. The main direction of translocation of 2,4-D and 2,4-DP is upwards (ie, away from the roots) in poplar. In ash there was no preferential direction of herbicide movement.

3. The resistance of ash is likely due to the lack of translocation of phytotoxic quantities of herbicide to the roots.

4. The sensitivity of poplar is likely due to translocation of phytotoxic quantities of one component of the mixture (2,4-D) to the roots.

Approved:

D. Harrison
Manager
Chemical Research Department

MKK:djb

Submitted:

M.K. Kirby
Biologist
Biological Research Section

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